COMPARATIVE ANALYSIS OF MALE BEHAVIOUR IN TWO SIBLING SPECIES *Microtus arvalis* Pall. AND *Microtus rossiaemeridionalis* Ogn. (RODENTIA, CRICETIDAE)

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Abstract. The analysis of open field and concurrent behaviour in males of *Microtus arvalis* and *Microtus rossiaemeridionalis,* inhabiting the central part of the Republic of Moldova was performed. The behaviour of males in *M. arvalis* and *M. rossiaemeridionalis* is complex and has intra- and interspecific patterns. Although the representatives of *M. rossiaemeridionalis* showed higher level of emotionality, this parameter cannot be used as criteria for species diagnosis. In dyadic encounters the males of studied species showed various elements of trial, aggressive, defensive, conflictual, friendly behaviour. Among the variety of behavioural reactions the most important are the elements connected with agonistic interactions, because it is well known the role of aggression in the mechanisms of number regulation of small mammal populations. It was established that the aggressiveness of intraspecific contacts had seasonal pattern and was higher than intraspecific one. At the same time, the intraspecific aggressiveness of the males was species-specific.

Keywords: Sibling species, open field, latent period, exploratory behaviour, dyadic encounters, agonistic relations.

Rezumat. Analiza comparativă a comportamentului masculilor speciilor sible *Microtus arvalis* Pall. şi *Microtus rossiaemeridionalis* Ogn. (Rodenţia, Cricetidae). A fost efectuată analiza comportamentului în câmp deschis și a relațiilor de concurență între masculii speciilor sible *Microtus arvalis* și *Microtus rossiaemeridionalis,* din zona centrală a Republicii Moldova. Comportamentul masculilor speciilor studiate este complex si prezintă particularități intra- și interspecifice. Deși reprezentanții *M. rossiaemeridionalis* au manifestat un grad mai înalt de emotivitate, acest parametru nu poate fi utilizat ca criteriu de identificare a speciilor. În așezările în cuplu masculii au manifestat diverse elemente comportamentale: de familiarizare, agresivitate, apărare, conflict, prietenoase. Printre varietatea de reacții etologice cele mai importante sunt elementele legate de interacțiunile antagoniste, deoarece este bine cunoscut rolul agresivității în mecanismele de reglare numerică a populațiilor de mamifere mici. S-a stabilit că agresivitatea în contactele intraspecifice prezintă variații sezoniere și a fost mai ridicată decât în contactele interspecifice. În același timp, agresivitatea intraspecifică la masculi avea caracter specio-specific.

Cuvinte cheie: specii sible, câmp deschis, comportament de explorare, așezări în cuplu, relații antagoniste.

INTRODUCTION

The sibling species *Microtus arvalis* Pallas, 1778 and *Microtus rossiaemeridionalis* Ognev, 1924 are closely related species externally indistinguishable, but genetically different (2n = 46 in *M. arvalis* and 2n = 54 in *M. rossiaemeridionalis*). The species are abundant and widespread in the temperate zone of Eurasia with largely overlapping ranges. Although, both species are extremely similar morphologically and ecologically and their settlements can be very close to each other, they are reproductively isolated (MEYER et al., 1972, 1996; MALYGIN, 1983). These species cannot be identified by standard morphological methods used in zoology. For their exact diagnosis molecular genetic methods and blood electrophoresis are used (MALYGIN, 1983; MEYER et al., 1996; MUNTEANU et al., 1999).

The behaviour and relationships between closely related species, such as the sibling vole species, are of particular interest, especially in terms of space use and maintenance of population structure (PANOV, 1975). The adaptive mechanisms of the species ensure the integrity and stability of the populations in the changing environmental conditions and contribute to the maintenance of a dynamic equilibrium between population and environment. The relatively stable existence of a population in time and space is strongly conditioned by the ethological structure, especially by the distribution of individuals and relationships between them (CHITTY, 1977; BUJALSKA & SAITOH, 2000).

In many studies marked differences in open-field behaviour were found between wild small rodents, but these studies were conducted mainly on *Apodemus* and *Mus* genus species (DOICHEV, 1983; ATANASOV, 1983; SOKOLOV et al., 1990; FRYNTA, 1992; SIMEONOVSKA-NIKOLOVA, 2000; TIKHONOVA et al., 2005; MUNTEANU et al., 2009; LARION, 2011; CEMIRTAN et al., 2013, 2014 etc.). Only scarce data are available for the comparison between sibling species *M. arvalis* and *M. rossiaemeridionalis* (MALYGIN, 1983; MUNTEANU & CEMÎRTAN, 2005; ECCARD & HERDE, 2013; CEMÎRTAN et al., 2014). The studies demonstrated obvious interspecific differences and also ecological correlates of this behaviour were found suggesting that open-field behaviour reflects ecological and selective pressures. As to the relationships between the individuals, many researchers consider intra- and interspecific competition among the most important factors determining the spatial distribution, daily activity, reproductive success in vole species (SOKOLOV & KUZNETSOV, 1978; DIENSKE, 1979; MALYGIN, 1983; DE JONGE, 1983; ZORENKO, 1984; TIKHONOV et al., 2009; TIKHONOVA et al., 2003, 2006; etc.).

For the study, there were chosen the males, because they have larger home ranges (BARANOVSKII & OHOTSKII, 1988), sometimes including several female's home ranges (KASATKIN, 2002; GROMOV 2008), therefore their spatial ability are greater than that of females (MALYGIN, 1983). The dispersal in voles is male-biased,

because females benefit more than males from staying on their natal patch (GREENWOOD, 1980), while the males are more active and mobile (DOBSON, 1982; PUSENIUS & VIITALA, 1993; GAUFFRE et al., 2009 etc.). The open-field behaviour and the intra- and interspecific relations of the males in dyadic encounters was studied in order to emphasize the behavioural traits of their identification, to elucidate the importance of exploratory behaviour and of intra- and interspecific relations of the males in population functioning of the studied species.

MATERIALS AND METHODS

The voles were collected from alfalfa crop (*M. arvalis*) and from the adjacent shelter belts (*M. rossiaemeridionalis*) in the central part of the republic during the whole year. In open field tests, 89 adult males (61 of *M. arvalis* and 28 of *M. rossiaemeridionalis*) were studied. The species identification was performed by blood electrophoresis method (MUNTEANU et al., 1999). Before the experiments the voles were weighed, the mean weight of *M. arvalis* males was 19.98 ± 7.62 g and that of *M. rossiaemeridionalis* was 20.5 ± 8.17 g. Each animal was individually tested for 15 min (five series of 3 min intervals each) in the open field experiment (HUGHES, 1987). The experiments were performed in a Plexiglas box with the dimensions 50 x 5 x 60 cm. The bottom was demarcated into 25 squares of equal dimensions (10 x 10cm) painted by dark lines. Mice were tested during their active phase in the morning and in the evening. The data from the open field tests were registered in protocols by shorthand. The following parameters were registered: latency period (time necessary for the animal to enter from the portable cage into the open field), horizontal activity (number of crossed squares), vertical activity (included the rear up on hind legs and jumps), grooming (self-cleaning), freezing (the animals stop moving and freeze usually in a corner), emotionality (defecation and urination).

The agonistic behaviour was tested (GOLTSMAN et al., 1977) by dyadic encounters within a box made of transparent Plexiglas (60 × 30 × 45 cm). The duration of every encounter was 15 min (three series of 5 min intervals each). The encounters were conducted between 9:00 a.m. and 11:00 a.m. A total of 64 adult males (42 of *M. arvalis* and 22 of *M. rossiaemeridionalis*) participated in tests; 74 dyadic encounters were run: 32 male-male of *M. arvalis*, 19 male-male of M. *rossiaemeridionalis* and 23 male-male between the species. The following parameters were registered: ratio of exploratory activity, number of contacts, type of contacts, number and ratio of agonistic contacts.

The statistical analysis and graphical representation was performed in Excel and Statistica programs, in comparison between data series the Student test was used, in order to emphasize the significance between the studied species.

RESULTS AND DISCUSSIONS

Open field test.

The latency period is the time that animal needs to overcome its fear and to enter in the open field from the portable cage. Among the tested voles very suspicious individuals were registered, who did not leave the portable cage within 10 minutes and were transferred in open field by the experimenter. More curious and fearless individuals left the portable cage and entered in open field by themselves within 10 minutes period. The ratio of suspicious and fearless individuals was slightly different: in *M. arvalis* – 9.7%, in *M. rossiaemeridionalis* – 14.3%. The mean value of latent period in curious males of field vole constituted 86.6 ± 13.4 sec., and 84.54 ± 10.25 sec. in East European vole, the differences between species being insignificant ($p > 0.1$).

Horizontal activity reflects the reaction of animal toward novelty, its ability to explore the environment and to adapt to it. The behaviour of both species was similar: in the first three minutes, the animal activity was the highest, toward the 6th minute it decreased significantly, and then the graduate decrease to the minimum values occurred (Fig. 1). Thus, the *M. rossiaemeridionalis* males reacted more actively toward novelty (the number of crossed squares in the first 3 minutes was 161 ± 17.1 against 119.47 ± 9.35 in *M. arvalis*, $p < 0.05$) and adapted quicker to it (38.9±5.1 and 26.5±4.38, respectively in the last 3 minutes of the test). On the whole, the parameter values of the first minutes in open field showed high level of animal emotional reaction to novelty and their decreasing proved the ability to overcome the fear and adapt to new environment.

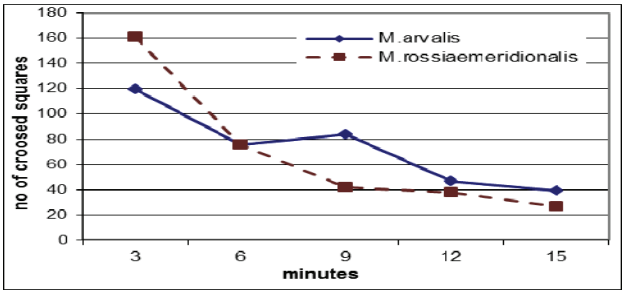

Figure 1. Horizontal activity of males in *M. arvalis* and *M. rossiaemeridionalis* during 15 minutes.

Vertical activity parameter was formed by two elements: rearing and jumping. The rearing activity was considered as proper exploratory activity, while the jumping was considered as emotional reaction toward new environment. The highest

levels of exploratory activity were recorded in the first 3 minutes, then it graduate decreasing occurred and reached minimum values in the last 3 minutes of the test (Fig. 2A). The dynamics and the values of the parameter were close in both species, as well as the total activity. Therefore, we can assume that both species have similar potential of exploratory activity.

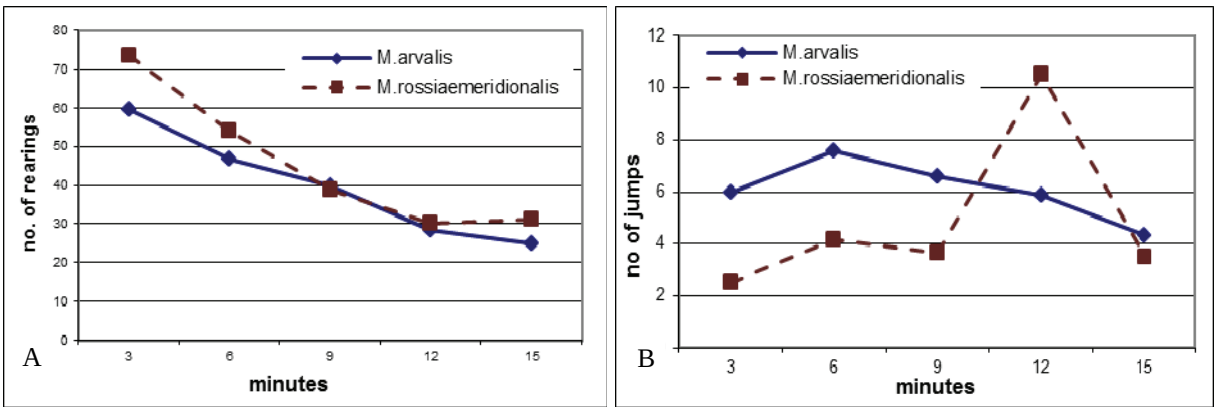

Figure 2. Vertical activity of males in *M. arvalis* and *M. rossiaemeridionalis* during 15 minutes and total vertical activity (A-rearing, B-jumping).

The vertical jumping on all 4 paws is an element particular for both species, but its values and dynamics was species-specific (Fig. 2B): in *M. arvalis* during all 3 minute periods the number of jumps varied insignificantly and were practically at the same level (5.97 ± 1.02 in the first 3 min. and 4.32 ± 0.99 in the last minutes). In *M. rossiaemeridionalis* this parameter was similar in the first 9 min. (2.5 ± 0.77 in the first 3 min., 3.67 ± 0.95 on the 9th min.), while on the 12th min. it raised to 10.5 ± 2.01, and at the end of the 15th min. it decreased to 3.5 ± 1.1. The mentioned results allow to conclude that *M. rossiaemeridionalis* males are more emotional than *M. arvalis.*

The grooming in males of both species increased toward the end of test. In *M. arvalis* this parameter increased from 10.32 ± 2.01 sec. to 49.03±5.38 sec. and in M. *rossiaemeridionalis* it increased from 5.5 ± 1.07 sec. to 50.67 ± 7.89 sec. (Fig. 3).

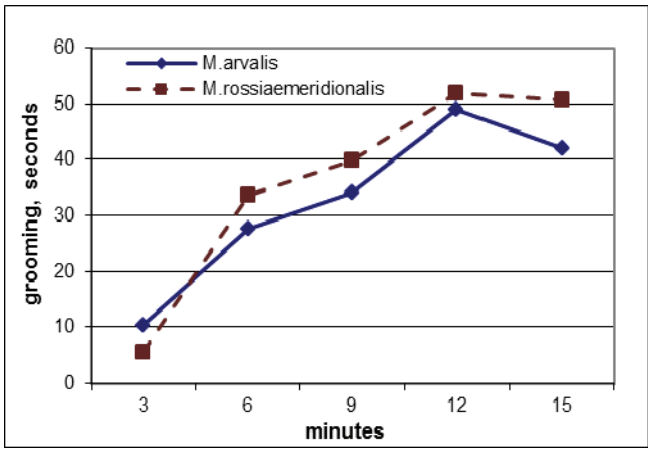

Figure 3. Grooming duration of males in *M. arvalis* and *M. rossiaemeridionalis* during 15 minutes.

Freezing is the period of voles' inactivity and was measured in seconds. Usually, the dynamics of this parameter is opposite to the dynamics of horizontal and vertical activities, thus with increasing of time spent in open field chamber the duration of inactivity periods also increased. During the test in both species the duration of motor and exploratory activities decreased and the freezing period increased, reaching maximum values in the last minutes of the test. In males of both species the dynamics of this parameter was similar (Fig. 4).

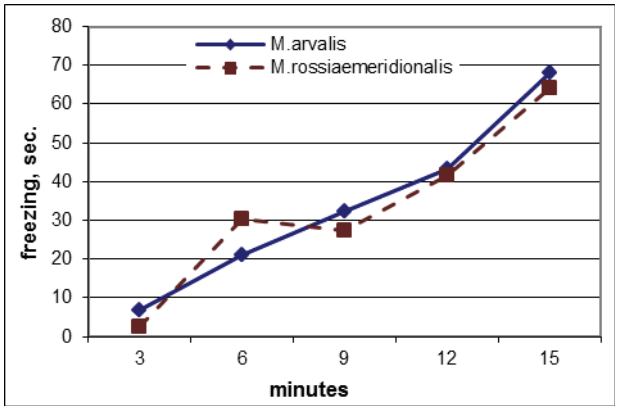

Figure 4. Freezing duration of males in *M. arvalis* and *M. rossiaemeridionalis* during 15 minutes.

Emotionality was calculated by counting the excrement and urination elements, thus establishing the vegetative component of emotional reaction. The urination element is not very particular for the voles, while the defecation occurred rather often at the majority of studied animals (Fig. 5). There were registered species-specific differences in this parameter: the emotionality in *M. arvalis* males was significantly lower ($p < 0.05$) than in *M. rossiaemeridionalis* males.

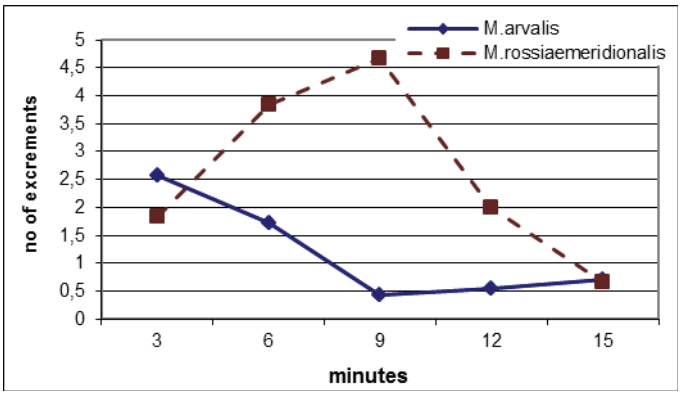

Figure 5. Emotionality of males in *M. arvalis* and *M. rossiaemeridionalis* during 15 minutes.

Dyadic encounters.

Since it is known that the voles form mixed settlements and thus maintain reproductive isolation, it was interesting to examine the nature of their interactions with each other. Using the method of dyadic encounters, we have studied the behaviour of males in intraspecific and interspecific pairs. In these studies, stereotypic behaviour was identified in voles, as well as their species-specific features.

In the first minutes of the test the voles began to explore the environment and in total amount of behavioural reaction the orientation-exploratory activity occupied one of the first places (Table 1). It was expressed in the free movement of animals within the camera and sniffing, sometimes licking the corners, touching, exploration of the walls and of the floor. At the same time attempts of individuals to become familiar with each other occurred, and the initiative of contact usually came from one of the partners. In such contacts different types of olfactory contact were observed: naso-nasal, naso-lateral, naso-dorsal, naso-ventral. The contacts could end by an attack, fled of one of the partners, mutual friendly contact and peaceful separation on opposite corners. Often passive staying of one or both animals at the corners of the chamber was observed, peaceful sitting side by side, grooming, allogrooming, sometimes mutual or one after another. During friendly contacts the animals crowded together in a corner, scrambled under or above each other, climbed over each other, often changing places.

The most diverse elements were associated with agonistic interactions, including assault, fight, chase, escape without attack and after it, pushing and boxing, aggressive stands, squeaks. In this group of interactions, there can be also included the contact avoiding with the partner, visual pursuit after the actions of the partner from a corner, as well as relations of domination and subordination, in which the subordinate animal lay on his back, to avoid the aggressive contact with the dominant, bowed his head in front of him, let the dominant to climb on it, to sit on its back or to be stomped etc. After the functional significance the described elements were combined in several behavioural groups: trial, aggressive, protective, conflictual, friendly behaviour (CEMÎRTAN et al., 2011, 2012).

Trial behaviour was expressed in the initiation of the contact, various types of olfactory contacts, exploratory behaviour.

Aggressive behaviour constituted 12-16% from the total amount of intraspecific contacts and about 10% from the total amount of interspecific ones (Table 1) and included attacks, fights, chase that sometimes finished with winning

over the partner (it laid down on its back and the chase was over), occupation of the territory, aggressive stands. The sequence and the set of antagonistic behaviour elements were varied: attack-fight-chase-winning; attack-aggressive stand-boxing-fight; attack-fight-occupation; attack-aggressive stand-chase, etc.

Defensive behaviour: running before or after attack, boxing, watching the partner in order to maintain the safe distance from it, pushing, squeak, freezing, subordination postures (lying on its back, head bowing in front of the partner).

Conflictual behaviour was expressed in ignoring the contact initiation, domination-subordination, grooming, allogrooming. The contact ignoring was expressed in that one of the animals at the initiative of contact from the other, continued to commit the same acts that occurred prior to this. At the superiority of one of the partners the relations of domination-subordination were observe. In this case the subordinate animal allows be trampling by the dominant, walking, sitting on itself, and when attempted to escape the dominant tried to keep the subordinate by force. We distinguish these elements from the above mentioned subordinate postures that occur during the aggressive contacts.

Friendly behaviour is formed by the following elements: going after the partner, getting together, climbing above, under, over the partner, allogrooming. Allogrooming was assigned, on the one hand, to conflictual behaviour, because there might have occurred relations of domination-subordination, and sometimes even aggressive grooming was observed: the dominant animal was biting while cleaning the subordinate. On the other hand, with equal partners the allogrooming is an element of friendly behaviour. The grooming was assigned to conflictual behaviour because it often represents a manifestation of shifted activity. At the same time it can be an element of comfortable behaviour.

Table 1. Dyadic encounters in males of sibling species *M. arvalis* and *M. rossiaemeridionalis.*

Parameter	**♂vs♂ *M. arvalis***	**♂vs♂ *M. rossiaemeridionalis***	**♂*M. arvalis* vs ♂*M. rossiaemeridionalis***
No of voles	42	22	64
No of tests	32	19	23
Exploratory activity	43.17%	41.26%	36.22%
Mean no of contacts	12.4±2.79	16.7±3.26	9.8±3.69
Ratio of agonistic contacts	22.4%	23.7%	12.6%

All the mentioned elements occurred in every dyadic encounter. Among the variety of behavioural reactions the elements associated with agonistic interactions are the most important, because it is known the role of aggression in the mechanisms of regulation of small mammal populations (SHILOV, 1977; MANTEIFEL, 1987). We assumed the implication of aggression in the mechanisms of maintaining the reproductive isolation in sibling species, therefore, during the tests a special attention was given to the degree of aggression in contacts between the voles. Thus, in dyadic encounters of *M. arvalis* males the highest level of agonistic contacts was registered in summer period (in 100% of pairs the dominant individual was established), while in autumn the males of this species were less aggressive (only in 64% of encounters the dominant was established, in the rest of the cases the relationships were peaceful and mutual). In dyadic encounters between M. *rossiaemeridionalis* males higher level of concurrent relations was registered in comparison to *M. arvalis* (in 100% of encounters the dominant individual was established, which was the heavier male). The interspecific contacts of the males were characterized by lower level of aggressiveness than intraspecific ones. In summer only in 60% of encounters the dominant was established and this usually was *M. arvalis*, while in autumn – in 50% and in majority of cases this was *M. rossiaemeridionalis.*

Thus, the aggressiveness of intraspecific contacts had seasonal pattern and was higher than intraspecific one. At the same time, the intraspecific aggressiveness of the males was species-specific.

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CONCLUSIONS

The behaviour of males in *M. arvalis* and *M. rossiaemeridionalis* is complex and has intra- and interspecific pattern. Although the representatives of *M. rossiaemeridionalis* showed higher level of emotionality, this parameter cannot be used as criteria for species diagnosis.

In dyadic encounters the males of studied species showed various elements of trial, aggressive, defensive, conflictual, friendly behaviour.

Among the variety of behavioural reactions the most important are the elements connected with agonistic interactions, because it is well known the role of aggression in the mechanisms of number regulation of small mammal populations. It was established that the aggressiveness of intraspecific contacts had seasonal pattern and was higher than intraspecific one. At the same time, the intraspecific aggressiveness of the males was species-specific.

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